

## DESCRIPTION

## DETECTING ELEMENT AND DETECTION METHOD

## 5 TECHNICAL FIELD

The present invention relates to a detecting element and a detection method. More specifically, the present invention relates to a detecting element and a detection method employing a plurality of  
10 layers of flow of a fluid in a microchannel, for detecting a plurality of different substances in a specimen.

## BACKGROUND ART

15 An electrochemical technique is simple and costs little, and thus has been widely used as a biosensor using a product of a catalytic reaction such as a product of an enzymatic reaction. Recently, an array-type sensor provided with a plurality of  
20 microelectrodes has been developed with miniaturization of electrodes in a detecting unit (see Japanese Patent Application Laid-Open No. 2002-071620).

An array-type sensor is capable of acquiring  
25 two-dimensional positional information with respect to the same specimen and dynamic changes in substance distribution. Further, an array-type sensor may

perform multichannel detection through reactions of electrodes set in array with specific, different specimens (See Kenichi Kojima, Atsunori Hiratsuka, Hiroaki Suzuki, Kazuyoshi Yano, Kazunori Ikebukuro, and Isao Karube, "Analytical Chemistry" 2003, 75, p. 1116-1122).

Meanwhile, a measuring device for sampling and measuring in a microchannel is used as a microfluidic device. Niwa et al. have proposed an online biosensor including a microchannel and a thin layer channel in combination and have conducted a continuous quantitative analysis of a trace amount of a sample (Japanese Patent Application Laid-Open No. H11-083784).

However, those techniques alone hardly allow separation and detection of a plurality of detection target substances in an actual measurement sample (such as blood) in the same channel at the same time. For example, the measurement sample may be distributed to a plurality of channels to detect the detection target substances in the respective channels, but a required amount of the measurement sample increases. In the case where a trapping site for trapping a plurality of detection target substances in the specimen is provided in a channel, a reaction product of a catalytic reaction, which is derived from the detection target substances with a

catalyst, is often the same substance for some of the detection target substances. The product of a catalytic reaction diffuses in a liquid, and thus, from which detection target substance the product was produced was hardly identified with one detecting unit (such as an electrode).

#### DISCLOSURE OF THE INVENTION

The present invention has been made in view of the prior art, and an object of the present invention is therefore to provide a detecting element and a detection method capable of detecting efficiently and simultaneously a trace amount of a plurality of different substances in a specimen by: supplying a trace amount of the specimen containing a plurality of different substances into a substance trapping portion without dividing a channel of a detecting element employing the channel into a plurality of channels and without distributing the specimen thereinto; and forming in the channel a plurality of substance trapping portions having respectively different detection targets.

A first aspect of the present invention relates to a detecting element for detecting a plurality of different substances in a specimen, characterized by including: a channel provided on a substrate, which is capable of forming a plurality of layers of flow

of a fluid; and a plurality of different substance trapping portions provided in the channel, for trapping the plurality of different substances in the specimen, respectively, in which: the plurality of  
5 different substance trapping portions are provided separately in accordance with the plurality of layers of flow of a fluid to be formed; and the plurality of different substance trapping portions are each arranged to acquire independent information on each  
10 of the substances in the specimen through an action between the fluid and the trapped substance.

The detecting element preferably further includes a detecting unit for detecting the information.

15 The plurality of different substances in the specimen each preferably have a label, and the fluid flowing along the plurality of layers of flow is preferably a fluid acting on the label to discharge an active product.

20 The label is preferably a substance having a catalytic action, a substance having electrochemiluminescence, or a fluorescent substance.

A second aspect of the present invention relates to a detection method for detecting a  
25 plurality of different substances in a specimen, characterized by including the steps of: introducing the specimen into a channel having a plurality of

different substance trapping portions for specifically trapping the plurality of different substances, respectively, to trap the substances in the substance trapping portions; forming a plurality  
5 of layers of flow of a fluid in the channel; and switching and passing the fluid forming the plurality of layers of flow, to acquire independent information on each of the substances in the specimen through an action between the fluid and the trapped substance.

10 The present invention provides a detecting element and a detection method capable of detecting efficiently and simultaneously a trace amount of a plurality of different substances in a specimen by: supplying a trace amount of the specimen containing a  
15 plurality of different substances into a substance trapping portion without dividing a channel of a detecting element employing the channel into a plurality of channels and without distributing the specimen thereinto; and forming in the channel a  
20 plurality of substance trapping portions having respectively different detection targets.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic diagram showing an  
25 example of a structure of a biosensor according to present invention.

Fig. 2 is a schematic diagram showing substance

trapping portions in a microchannel of Example 1.

Fig. 3 is a schematic diagram showing a constitution of a PDMS substrate of Example 1.

Fig. 4 is a schematic diagram showing a  
5 constitution of a glass substrate of Example 1.

Fig. 5 is a schematic diagram of a reaction in the substance trapping portions of Example 1.

Fig. 6 is a schematic diagram of an electrode unit of Example 1.

10 Fig. 7 is a schematic diagram showing a structure of a biosensor shown in Example 2.

Fig. 8 is a schematic diagram of substance trapping portions in a microchannel of Example 2.

Fig. 9 is a schematic diagram showing a  
15 constitution of a microchannel on a glass substrate of Example 2.

Fig. 10 is a schematic diagram showing a constitution of through holes on the glass substrate of Example 2.

20 Fig. 11 is a schematic diagram of a reaction in the substance trapping portions of Example 2.

The reference numerals used in the drawings indicate the following portions:

	1, 2	inlet
25	3	drain
	4, 5, 6	electrode pad
	7	substrate

	9	microchannel
	10, 11	substance trapping portion
	13	PDMS substrate
	14	microchannel
5	15	glass substrate
	16, 17, 18	thin film electrode
	19, 20, 21	electrode pad
	22, 23, 24	through hole
	25	PDMS substrate
10	26	BSA
	27	rabbit antibody to AFP
	28	AFP
	29	mouse antibody to AFP
	30	goat antimouse antibody
15	31	GOX
	32, 33, 34	electrode pad
	35	microchannel
	36, 37, 38	inlet
	39	drain
20	40	substrate
	42, 43	substance trapping portion
	44, 45	interface
	46	subjected to hydrophobic treatment
	47	substrate
25	48	microchannel
	49	glass substrate
	50, 51, 52, 53	through hole

	54	PDMS substrate
	55	BSA
	56	rabbit antibody to AFP
	57	AFP
5	58	mouse antibody to AFP
	59	goat antimouse antibody
	60	HRP
	71, 72	laminar flow

#### 10 BEST MODE FOR CARRYING OUT THE INVENTION

Hereinafter, the present invention will be described in detail.

A detecting element of the present invention is a detecting element for detecting a plurality of  
15 different substances in a specimen, characterized by including: a channel provided on a substrate, which is capable of forming a plurality of layers of flow (hereinafter, referred to as laminar flows) of a fluid; and a plurality of different substance  
20 trapping portions provided in the channel, for trapping the plurality of different substances in the specimen, respectively, in which the plurality of different substance trapping portions are each arranged to acquire independent information on each  
25 of the substances in the specimen by switching the fluid forming the plurality of laminar flows.

Further, a detection method of the present



invention is a detection method for detecting a plurality of different substances in a specimen, characterized by including the steps of: introducing the specimen into a channel having a plurality of  
5 different substance trapping portions for specifically trapping the plurality of different substances; forming a plurality of laminar flows in the channel; and switching the fluid forming the plurality of laminar flows, to acquire independent  
10 information on each of the substances in the specimen.

A general mode of the present invention is a detecting element of a  $\mu$ TAS (Micro Total Analysis Systems) type represented in Fig. 1. As shown in Fig. 2, an antibody bonding specifically with a substance  
15 in a specimen is immobilized onto a substance trapping portion in the microchannel.

A plurality of substance trapping portions are formed in the channel to trap a plurality of substances in the specimen, respectively.

20 The substance trapping portions are each arranged to acquire independent information on each of the substances by switching a fluid forming a plurality of laminar flows in the channel. The fluid forming the laminar flows is switched for acting on a  
25 label of a substance in the specimen to pass a fluid (substrate) for discharging an active product through one layer alone, and the discharged active product is

detected, to thereby acquire information such as an amount of the substance in the specimen trapped in the substance trapping portion corresponding to the layer.

5        Examples of the label that can be used include a substance having a catalytic action, a substance having electrochemiluminescence, and a fluorescent substance. Further, a substance itself in the specimen may serve as a label. A substance in the  
10 specimen is detected by, for example: forming a complex of the specimen trapped in the substance trapping portions and an enzyme-labeled, substance-specific secondary antibody; selectively supplying an enzyme-substrate acting on the enzyme-label with a  
15 fluid forming laminar flows corresponding to the respective substance trapping portions; and detecting the produced enzymatic reaction product (active product) with a detecting electrode provided downstream of the substance trapping portions.

20        A target specimen may be one which can be specifically recognized by an antibody of a biological substance (protein, nucleic acid, sugar chain), allergen, bacteria, virus, or the like.

      The complex to be formed in the substance  
25 trapping portions may include a tertiary or higher enzyme-labeled antibody which specifically bonds with a complex of the specimen and the secondary antibody,

in addition to the enzyme-labeled secondary antibody.

Further, in the case where a fluorescent substance is used as a labeling substance, selective detection involves changing of pH of the laminar  
5 flows and selective spectrum shifting.

Examples of the substance having a catalytic action include glucose oxidase, choline oxidase, lactose oxidase, and horseradish peroxidase. Examples of a substrate therefor include glucose,  
10 choline, lactose, luminol, and hydrogen peroxide. An example of the substance having electrochemiluminescence includes tris(bipyridyl)ruthenium. Examples of the fluorescent substance include: 5-carboxyfluorescein;  
15 8-amino-2-(trans-2-aminostyryl)-6-methoxyquinoline-N,N,N',N'-tetraacetic acid, tetrasodium salt (Quene-1); and 2',7'-bis(carboxyethyl)-4(5)-carboxyfluorescein (BCECF).

The detecting element of the present invention  
20 allows integration of the substance trapping portions for detecting a plurality of substances in the specimen, and efficient detection of the specimen with a smaller amount. Further, the specimen need not be divided and passed through multichannels, and  
25 the specimen can be used as efficiently as possible.

Further, in a biosensor, the detection method allows selective detection of signals (enzymatic

reaction product, chemiluminescence, and fluorescence) from the respective substance trapping portions. Specific detecting units for the respective reactions are not required, and the  
5 detecting units can be shared. The detection method allows integration and sharing of the substance trapping portions and the detecting units, to thereby provide a microsensor at low cost.

Hereinafter, the present invention will be  
10 described in more detail based on examples with reference to drawings. However, the present invention is not limited to the following examples.

#### Example 1

Fig. 1 shows a structure of a biosensor  
15 according to an embodiment of the present invention. Glucose oxidase (GOX) was used as a substance having a catalytic action. Further, human  $\alpha$ -fetoprotein (AFP) and human  $\beta$ 2-microglobulin ( $\beta$ 2MG) were used as specimens of measuring objects.

20 The biosensor had a structure including a polydimethylsiloxane (PDMS) substrate and a glass substrate attached together. A rectangular microchannel 14 having a width of 100  $\mu$ m and a depth of 100  $\mu$ m was patterned on a PDMS substrate 13 (see  
25 Fig. 3). Thin film electrodes 16, 17, and 18 for detecting hydrogen peroxide and electrode pads 19, 20, and 21 were patterned on a glass substrate 15 (see

Fig. 4). The electrodes and the electrode pads were formed by laminating titanium and platinum through sputtering in the described order. The glass substrate was provided with through holes (diameter of 100  $\mu\text{m}$ ) 22, 23, and 24 used as two inlets 1 and 2 for injecting a specimen and one drain 3. A reference electrode 17 was plated with silver as a reference material.

As shown in Fig. 2, substance trapping portions 10 and 11 were provided in a microchannel, and a rabbit antibody to human AFP and a rabbit antibody to human  $\beta 2\text{MG}$  were cross-linked and immobilized on the substance trapping portions respectively with a glutaraldehyde vapor through bovine serum albumin (BSA). The PDMS substrate and the glass substrate were attached through autoadsorption. A phosphate buffered saline was used as a measuring solution, and was supplied from the two inlets 1 and 2 at a flow rate of 2  $\mu\text{l}/\text{min}$  each by a syringe pump.

Three electrode pads 32, 33, and 34 of a working electrode, a reference electrode, and a counter electrode aligned as shown in Fig. 6 were connected to terminals of a potentiostat, respectively. When a mixed solution of human AFP and human  $\beta 2\text{MG}$  as a specimen was supplied as the measuring solution from the two inlets, human AFP and human  $\beta 2\text{MG}$  were bonded to the substance trapping

portions through the respective immobilized antibodies. Next, secondary antibodies (mouse-derived) to human AFP and to human  $\beta$ 2MG were supplied thereto. Finally, a GOX-modified goat antimouse  
5 antibody was supplied thereto as a tertiary antibody, to thereby form a complex as shown in Fig. 5.

Then, the specimen was assayed. Human AFP and human  $\beta$ 2MG were trapped separately in the microchannel. Laminar flows 71 and 72 were formed in  
10 the microchannel to pass glutamic acid which is a GOX enzyme-substrate through the respective substrate trapping portions. A buffer solution containing glutamic acid was passed through only one of the two inlets 1 and 2, to thereby detect hydrogen peroxide  
15 generated from only one GOX of AFP trapping portion and  $\beta$ 2MG trapping portion as a reduction current by using the thin film electrodes 16, 17, and 18 provided downstream. Then, an inlet for introducing glutamic acid was switched, to thereby detect  
20 hydrogen peroxide generated from a different GOX. Such an operation allows separate detection of two specimens (AFP and  $\beta$ 2MG) trapped in one microchannel using the same label with one detecting unit.

#### Example 2

25 A biosensor having a structure as shown in Fig. 7 was prepared. A glass substrate was used as a substrate. As shown in Fig. 9, a microchannel 48

having a width of 100  $\mu\text{m}$  and a depth of 50  $\mu\text{m}$  was formed on a glass substrate 47 through a wet etching process. The laminar flows formed in Example 1 were detected more strictly, to thereby suppress diffusion of the substrate and form substance trapping portions more closely. In this way, the substance trapping portions can be formed at higher density.

In this example, three inlets were formed as shown in Fig. 8. A central inlet passed a solution used for detecting laminar flows more strictly. Thus, an organic solution is more desirable than an aqueous solution. In this example, ethanol was used. A surface of a microchannel connected to the central inlet was subjected to hydrophobic treatment just before a convergence 46 with other two channels, to thereby prevent backflow of a phosphate buffered saline flowing from the other two inlets. A fluorine compound ( $\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{CH}_2\text{Si}(\text{OMe})_3$ ) was used for the hydrophobic treatment and was coated onto the surface. Horseradish peroxidase (HRP) was used as a labeling enzyme.

Similar to Example 1, primary antibodies were immobilized on substance trapping portions 42 and 43 of Fig. 8. As shown in Fig. 10, through holes 50, 51, 52, and 53 for the inlets and a drain were formed on the glass substrate. The two glass substrates were attached together with an ultraviolet curing adhesive

by masking a microchannel portion to prevent ultraviolet exposure thereto. In the measurement of the specimen, a mixed solution of human AFP and human  $\beta$ 2MG was supplied from inlets 36 and 38 at a flow rate of 2  $\mu$ l/min by a syringe pump. Next, secondary antibodies (mouse-derived) to human AFP and human  $\beta$ 2MG were supplied thereto. Finally, an HRP-modified goat antimouse antibody was supplied thereto as a tertiary antibody, to thereby form a complex as shown in Fig. 11.

Then, the specimen was assayed. Human AFP and human  $\beta$ 2MG were trapped separately in the microchannel. Laminar flows were formed in the microchannel to pass luminol which is an HRP enzyme-substrate through the respective substance trapping portions 42 and 43. A phosphate buffered saline containing luminol and hydrogen peroxide was passed through only one of the two inlets 36 and 38. A phosphate buffered saline was passed through the other inlet. Further, ethanol was passed through the inlet 37 to separate two water phases and reduce diffusion of luminol. Luminol and hydrogen peroxide reacted through a catalytic action of HRP, to thereby form a 3-aminophthalate dianion and, at the same time, cause chemiluminescence. The specimen bonded to the substance trapping portion 42 or 43 was assayed by measuring a luminescence intensity.



Then, an inlet for introducing luminol and hydrogen peroxide was switched, to thereby detect chemiluminescence by different HRP. Such a procedure allows separate detection of two specimens (AFP and  
5  $\beta$ 2MG) trapped in one microchannel using the same label.

### Example 3

A fluorescent label can be used in place of the enzyme-label in the systems of Examples 1 and 2.

10 A pH of a fluid in a specific layer was changed by formation of laminar flows to change fluorescent characteristics of the fluorescent label, to thereby allow distinction of the specimens. The specimens were distinguished by detecting fluorescence  
15 intensities at a specific wavelength or changes in fluorescence spectra. Similar to Example 2, the specimen can be detected by using pH-sensitive fluorescent dye 5-carboxyfluorescein as a label for a tertiary antibody.

20

### INDUSTRIAL APPLICABILITY

The detecting element and detection method of the present invention allow efficient and simultaneous detection of a trace amount of a  
25 plurality of substances in a specimen, and thus can be used for a biosensor for medical diagnoses, medical examinations, food safety inspections,

environmental pollutant tests, or the like.

This application claims priority from Japanese  
5 Patent Application No. 2004-015974 filed January 23,  
2004, which is hereby incorporated by reference  
herein.

## CLAIMS

1. A detecting element for detecting a plurality of different substances in a specimen, characterized by comprising:

5 a channel provided on a substrate, which is capable of forming a plurality of layers of flow of a fluid; and

a plurality of different substance trapping portions provided in the channel, for trapping the  
10 plurality of different substances in the specimen, respectively, wherein:

the plurality of different substance trapping portions are provided separately in accordance with the plurality of layers of flow of a fluid to be  
15 formed; and

the plurality of different substance trapping portions are each arranged to acquire independent information on each of the substances in the specimen through an action between the fluid and the trapped  
20 substance.

2. A detecting element according to claim 1, characterized by further comprising a detecting unit for detecting the information.

3. A detecting element according to claim 1 or  
25 2, characterized in that:

the plurality of different substances in the specimen each have a label; and

the fluid flowing along the plurality of layers of flow comprises a fluid acting on the label to discharge an active product.

4. A detecting element according to claim 3,  
5 characterized in that the label comprises a substance having a catalytic action, a substance having electrochemiluminescence, or a fluorescent substance.

5. A detecting element according to claim 4,  
wherein the substance having a catalytic action is  
10 selected from glucose oxidase, choline oxidase, lactose oxidase, and horseradish peroxidase.

6. A detecting element according to claim 1,  
characterized in that an antibody is immobilized on each of the substance trapping portions.

15 7. A detection method for detecting a plurality of different substances in a specimen, characterized by comprising the steps of:

introducing the specimen into a channel having a plurality of different substance trapping portions  
20 for specifically trapping the plurality of different substances, respectively, to trap the substances in the substance trapping portions;

forming a plurality of layers of flow of a fluid in the channel; and

25 switching and passing the fluid forming the plurality of layers of flow, to acquire independent information on each of the substances in the specimen

through an action between the fluid and the trapped substance.

8. A detection method according to claim 7,  
wherein the plurality of different substances in the  
5 specimen each have a label.

9. A detection method according to claim 8,  
wherein the label employs a substance having a  
catalytic action, a substance having  
electrochemiluminescence, or a fluorescent substance.

10 10. A detection method according to claim 7,  
wherein the plurality of different substances in the  
specimen are selected from a biological substance,  
allergen, bacteria, and virus.

11. A detection method according to claim 10,  
15 wherein the biological substance is selected from a  
protein, a nucleic acid, and a sugar chain.

## ABSTRACT

A detecting element for detecting a plurality of different substances in a specimen, characterized by including: a channel provided on a substrate, 5 which is capable of forming a plurality of layers of flow of a fluid; and a plurality of different substance trapping portions provided in the channel, for trapping the plurality of different substances in the specimen, respectively, in which: the plurality 10 of different substance trapping portions are provided separately in accordance with the plurality of layers of flow of a fluid to be formed; and the plurality of different substance trapping portions are each arranged to acquire independent information on each 15 of the substances in the specimen through an action between the fluid and the trapped substance.

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FIG. 1

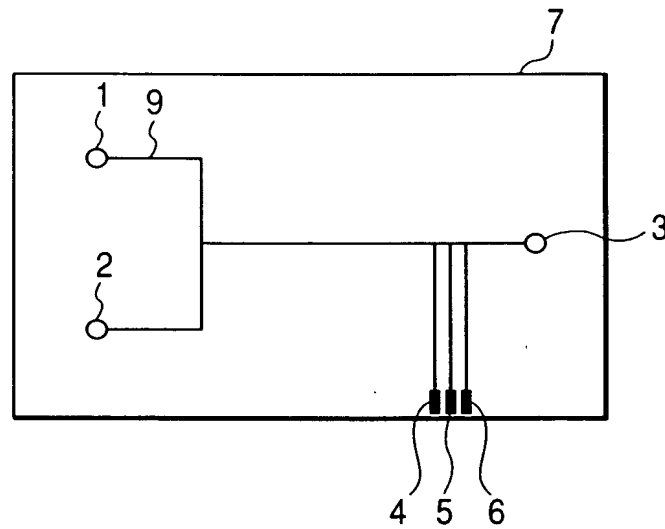
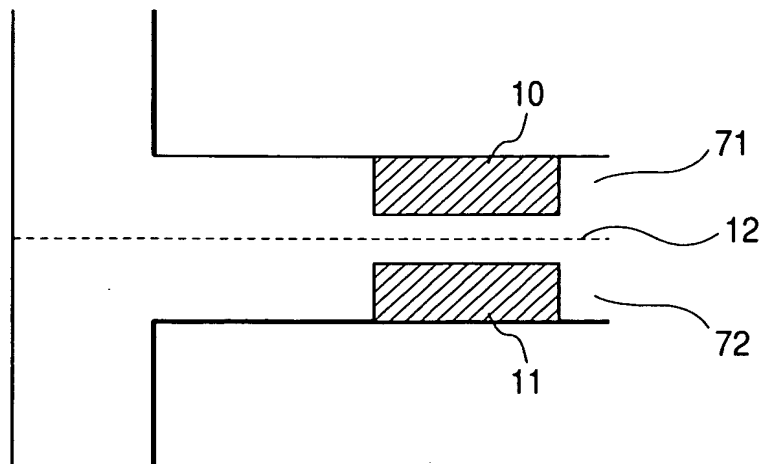


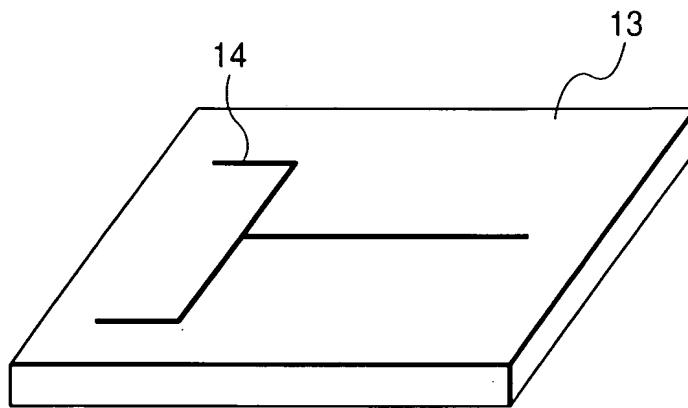
FIG. 2



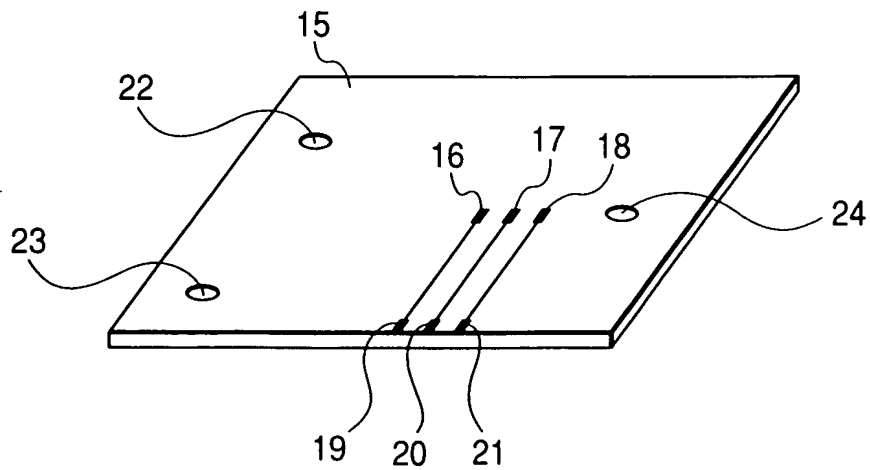
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**FIG. 3**



**FIG. 4**

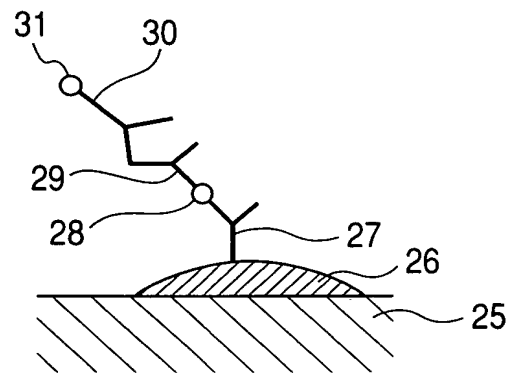




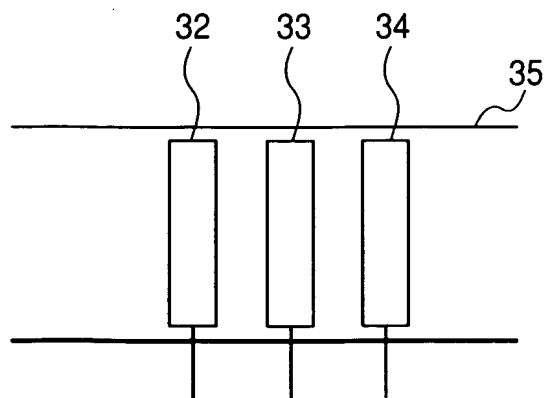
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**FIG. 5**



**FIG. 6**



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FIG. 7

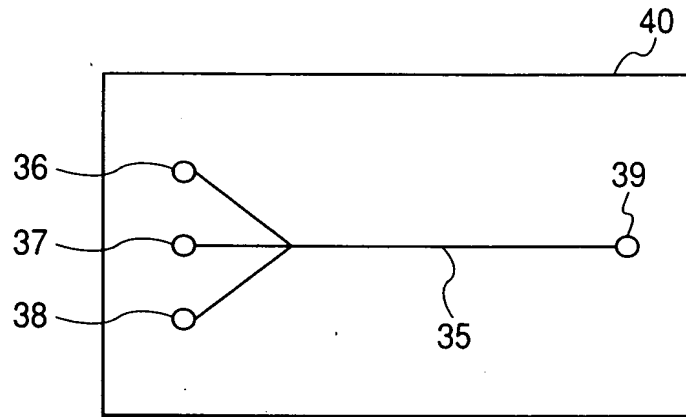
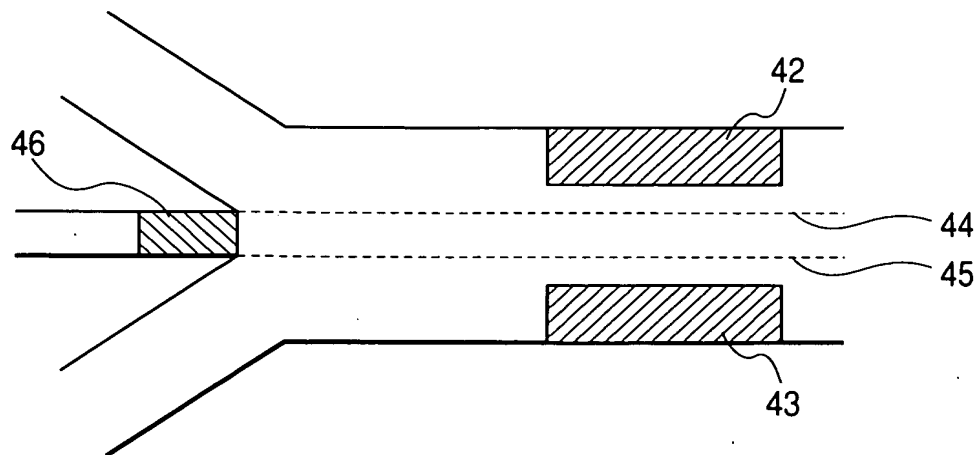


FIG. 8



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FIG. 9

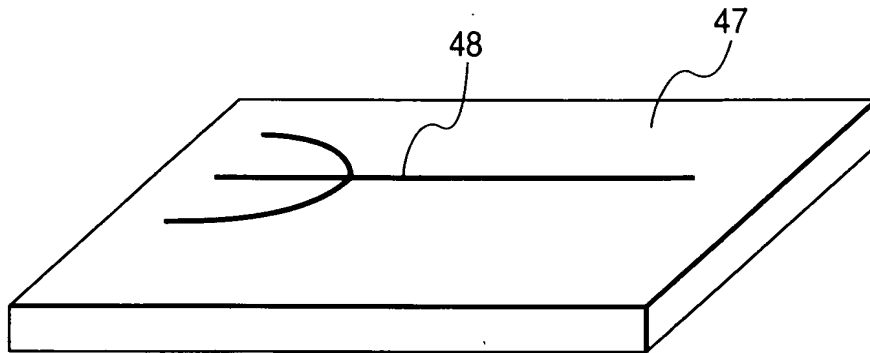
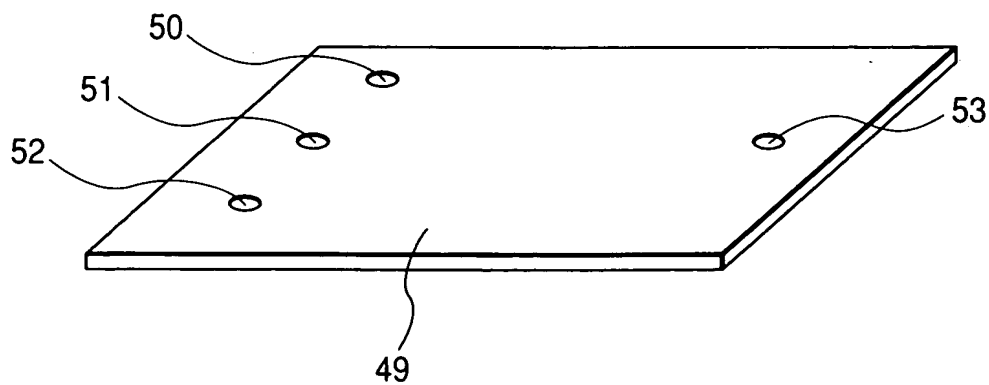


FIG. 10



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**FIG. 11**

